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(54) Title: ANTI-INFECTIVE ECTA™

(57) Abstract: The present invention provides compositions and methods for targeting toxic anti-metabolites to inhibit the growth of antibiotic resistant microbial infections. It provides a means of taking advantage of a key disease resistance mechanism to activate these drugs locally, and to overcome the resistance phenotype of the microbes. In addition, the invention provides methods for treating a subject infected with an antibiotic resistant microorganism by administering the compounds or compositions of the invention.

ANTI-INFECTIVE ECTA™

CROSS-REFERENCE TO RELATED APPLICATIONS

5 This application claims priority under 35 U.S.C. § 119(e) to U.S. Provisional Application No. 60/185,479, filed February 28, 2000, the contents of which are hereby incorporated by reference into the present disclosure.

TECHNICAL FIELD

10 The present invention relates to the field of Enzyme Catalyzed Therapeutic Activation (ECTA™) therapy and specifically to substrates of enzymes which are expressed by infectious agents and which thereby block efficacy of currently available drugs.

BACKGROUND

15 Throughout this disclosure, various publications are referenced by first author and date, within parentheses, patent number or publication number. The complete bibliographic reference is given at the end of the application, immediately preceding the claims. The disclosures of these references are hereby
20 incorporated by reference into this disclosure to more fully describe the state of the art to which this application pertains.

 Resistance to antimicrobial agents is a recognized medical problem (Schaechter et al., 1993; Murray, 1997). The problem was recognized early as penicillin resistance in Staphylococci, and is now a recognized problem for the
25 treatment of many bacterial infections, including essentially all nosocomial (hospital-acquired) bacterial infections (Bush, 1988; Steinberg et al., 1996; Murray, 1997). Nosocomial infections occur in 5% of patients admitted to the

hospital (about 2 million patients per year in the United States); they cause an estimated 20,000 deaths per year, and contribute to an additional 60,000 hospital deaths. It is estimated that nosocomial infections add about 7.5 million hospital days and \$1 billion dollars in health care costs each year (Wilson et al., 1991).

- 5 The importance of antibiotic resistant bacteria has increased as many organisms, e.g., *Staphylococcus aureus*, have developed resistance to several distinct antibiotics (the "multi-resistant" phenotype). The enzymes involved in drug resistance include the penicillinases, β -lactamases, cephalosporinases, and others. These enzymes inactivate antibiotics by modifying them to inactive compounds.
- 10 Resistance caused by enzymes also includes antibiotic modification by choramphenicol acetyltransferases and other aminoglycoside modifying enzymes (Murray, 1997). Other mechanisms which contribute to antibiotic resistance include drug permeability mutations, expression of transport proteins that actively extrude antibiotics from target organisms, and mutations in the drug targets
- 15 themselves (Murray, 1997).

Characteristics of Antibiotics

- Antibiotics are drugs that have cytostatic or cytotoxic effects on target organisms. The key to success for an antibiotic is selectivity for the disease target,
- 20 and lack of toxicity to the host, or patient. Many antibiotics are purified from cultures of microbial organisms themselves, while others are synthetic derivatives of naturally produced antibiotics (Wilson, et al., 1991). The most useful antibiotics against infections are those which attack a microbe-specific target. For example, β -lactam antibiotics interfere with cell wall synthesis by binding to cell
- 25 wall precursors. Since mammalian cells lack the cell walls of bacteria, these drugs have a tremendous margin of safety for the patient. The most common form of resistance to β -lactam antibiotics is the production of β -lactamases, which degrade the antibiotic molecule. The β -lactamases are encoded by either plasmid or chromosomal genes.

- 30 Although inactivation of antibiotics is probably the most common mechanism for drug resistance, resistance also occurs as a result of mutations in the drug targets themselves. The best characterized of these are mutations in the

penicillin-binding-proteins (PBPs), leading to a decrease or loss in the binding of antibiotics by these proteins and a corresponding decrease or loss in antibiotic activity. The β -lactam antibiotics include penicillin, ampicillin, carbenicillin, and the cephalosporins (including cephalexin, cefaclor, cefoxitin, cefotaxime and cefoperazone). Because resistance is very common via production of high levels of β -lactamases, new drugs have been developed to inhibit these enzymes, thereby increasing the efficacy of the β -lactam antibiotics. Examples of β -lactamase inhibitors include clavulanate, timentin and sulbactam (Bush, 1988; Wilson, et al., 1991; Schaechter, et al., 1993). The combination of β -lactam antibiotic with β -lactamase inhibitor has extended the useful pharmacologic lifetimes of these antibiotics (Bush, 1988).

Drawbacks of current antimicrobial agents

Current agents have well characterized targets of action. Several examples are given below:

<u>Antibiotic Family</u>	<u>Example</u>	<u>Target</u>
β -lactam antibiotics	Penicillins, cephalosporins	Cell wall biosynthesis
Sulfonamides	Sulfanilamide	Blocks synthesis of tetrahydrofolate
Aminoglycosides	Streptomycin	Protein synthesis
Trimethoprim	---	Folate metabolism
Chloramphenicol	---	Protein synthesis
Vancomycin	---	Cell wall synthesis

Other antibiotics work by blocking DNA replication, production of cellular RNA, or by modification of multiple cellular targets (Schaechter, et al., 1993). The occurrence of resistance to antibiotics is commonplace, and many of the mechanisms have been described (Schaechter et al., 1993; Murray, 1997). These mechanisms include overexpression of the target enzyme, expression of an

antibiotic inactivating enzyme, or mutation of the target so that it is no longer recognized by the antibiotic. Examples of these are given below:

<u>Antibiotic</u>	<u>Principle mechanism of resistance</u>
Penicillins and other β -lactam Antibiotics	Inactivation by β -lactamase
Sulfanilamide	Mutation of dihydropteroate synthase target enzyme
Aminoglycosides	Inactivation by aminoglycoside modifying enzyme, or by target mutation
Trimethoprim	Mutation of dihydrofolate reductase target enzyme
Chloramphenicol	Inactivation by chloramphenicol transacetylase
Methicillin	Mutation of penicillin binding proteins
Vancomycin	Mutation in target cell wall peptide

Increased resistance of bacterial infection to treatment with antibiotics has
5 been carefully documented (see, e.g., Steinberg et al., 1996), and has now become
a generally recognized problem (Murray, 1997). Each "new" antibiotic derived
from its previous generation (e.g., cephalosporin from penicillin) is initially
successful, but then has had increasing reports of resistance. The progression of
 β -lactamases antibiotics is typical of the field. Each successive antibiotic is more
10 resistant to degradation by β -lactamase, and the organism then produces larger
amounts of the β -lactamase. This is especially a problem for nosocomial (hospital
acquired) infections (Wilson et al., 1991; Murray, 1997). The most common
mechanism for transmission of the drug resistance phenotype is via plasmids,
although some modulators of antibiotic resistance are located on the bacterial
15 chromosome (Schaechter et al., 1993). The despair of the medical community has
been addressed by the production of inhibitors of the β -lactamases.
Unfortunately, although the β -lactamases have overlapping substrate specificities,
they have evolved differently to have distinct, but related, amino acid sequences.

This problem is expressed by the widely varying efficacies of each β -lactamase inhibitor for different enzymes.

Vancomycin inhibits synthesis and assembly of the second stage of cell wall peptidoglycan polymers by complexing with their D-alanyl-D-alanine precursor, which fits into a "pocket" in the vancomycin molecule, thereby preventing its binding to the peptidoglycan terminus that is the target of transglycolase and transpeptidase enzymes. In addition, vancomycin may impair RNA synthesis and injure protoplasts by altering the permeability of their cytoplasmic membrane. Vancomycin-resistant enterococci (VRE) emerged as important nosocomial pathogens in the United States. Strains of *S. aureus* that were intermediately resistant to vancomycin (VIRSA) were detected in the United States in 1997. VRE and VIRSA have raised serious concerns about the continued effectiveness of vancomycin in the treatment of these infections. Vancomycin-resistant enterococci produce two new enzymes, a ligase and a dehydrogenase, with formation of a new depsipeptide terminus D-ala-D-lactate, to the pentapeptide. This substitution allows cell wall synthesis to continue in the presence of the vancomycin.

The new generation antibiotics are usually more toxic than their predecessors, and cannot be administered to patients in a convenient way. A cycle of drug resistance has been established which requires a new approach to resolve. Therefore, a need exists for a new generation of antibiotics that are not susceptible to the established drug-evasion mechanisms. This invention satisfies this need and provides related advantages as well.

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DISCLOSURE OF THE INVENTION

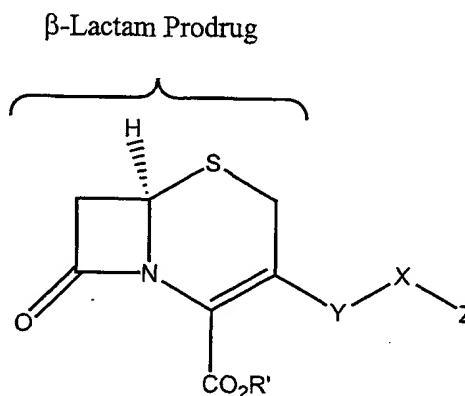
Many enzyme-prodrug combinations have been described in detail. Applications have included antiviral drugs like ganciclovir (Straus, 1993) and antibody or gene directed expression of bacterial enzymes to treat cancer (Melton and Sherwood, 1996; Stosor et al., 1996). The current invention redirects this technology to treat infectious disease resistant to therapy by antibiotics.

Thus, this invention provides prodrugs and methods for selectively inhibiting the proliferation of antibiotic resistant microorganism by contacting a

sample containing such a microorganism with an effective amount of these prodrugs. In addition, the invention provides methods for treating a subject infected with an antibiotic resistant microorganism by administering the compositions of the invention.

5

The prodrugs of this invention have the general structure shown below.



10

X, Y, Z, and R' are specifically defined throughout this application.

MODES FOR CARRYING OUT THE INVENTION

As used herein, certain terms may have the following defined meanings.

The singular form "a," "an" and "the" include plural references unless the context clearly dictates otherwise. For example, the term "a cell" includes a plurality of cells, including mixtures thereof.

The term "comprising" is intended to mean that the compositions and methods include the recited elements, but not excluding others. "Consisting essentially of" when used to define compositions and methods, shall mean excluding other elements of any essential significance to the combination. Thus, a composition consisting essentially of the elements as defined herein would not exclude trace contaminants from the isolation and purification method and pharmaceutically acceptable carriers, such as phosphate buffered saline,

preservatives, and the like. "Consisting of" shall mean excluding more than trace elements of other ingredients and substantial method steps for administering the compositions of this invention. Embodiments defined by each of these transition terms are within the scope of this invention.

5 As used herein the term "prodrug" means a precursor or derivative form of a pharmaceutically active agent or substance that is less cytotoxic to a target cell as compared to the drug metabolite and is capable of being enzymatically activated or converted into the more active form.

10 A "composition" is intended to mean a combination of active agent and another compound or composition, inert (for example, a detectable agent or label or a pharmaceutically acceptable carrier) or active, such as an adjuvant.

A "pharmaceutical composition" is intended to include the combination of an active agent with a carrier, inert or active, making the composition suitable for diagnostic or therapeutic use *in vitro*, *in vivo* or *ex vivo*.

15 As used herein, the term "pharmaceutically acceptable carrier" encompasses any of the standard pharmaceutical carriers, such as a phosphate buffered saline solution, water, emulsions, such as an oil/water or water/oil emulsion, and various types of wetting agents. The compositions also can include stabilizers and preservatives. For examples of carriers, stabilizers and adjuvants,
20 see Martin, REMINGTON'S PHARM. SCI., 15th Ed. (Mack Publ. Co., Easton (1975)).

An "effective amount" is an amount sufficient to effect beneficial or desired results. An effective amount can be administered in one or more administrations, applications or dosages.

25 A "control" is an alternative subject or sample used in an experiment for comparison purposes. A control can be "positive" or "negative".

An antibiotic resistant microorganism is a microorganism with the ability to diminish or inhibit the ability of the antibiotic to inhibit the growth or kill the microorganism.

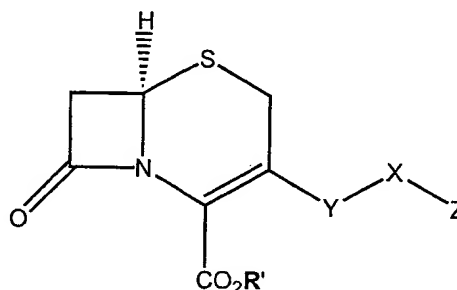
30 A " β -lactam resistant microorganism" is a microorganism with the ability to synthesize a protein that neutralizes a β -lactam antibiotic.

"Inhibiting the growth" of a microorganism means reducing by contact with an agent, the rate of proliferation of such a microorganism, in comparison with a control microorganism of the same species not contacted with this agent.

A "subject" is a plant or a vertebrate such as a fish, an avian or a mammal, and preferably a human. Fish include, but are not limited to pets and farm animals. Avians include, but are not limited to pets, sport animals and farm animals. Mammals include, but are not limited to, murines, simians, humans, farm animals, sport animals, and pets.

The present invention provides compositions and methods for targeting toxic anti-metabolites to antibiotic resistant microbial infections. In one embodiment, the invention provides a means of taking advantage of a key disease resistance mechanism, for example, the overproduction of β -lactamase enzyme, to activate these drugs locally, and to overcome the resistance phenotype and inhibit the growth of the microbes. The invention further provides methods for treating a subject infected with antibiotic resistant microorganism by administering an effective amount of the compounds or compositions of the invention.

In one aspect, the invention provides a prodrug compound having the structure:



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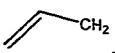
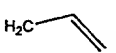
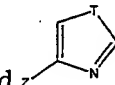
wherein R' is selected from the group consisting of hydrogen, alkyl, aryl, halogenated aryl, phenol, nitro aryl, ammonium, methylamine, dimethylamine, lower alkylamine, bis (lower alkyl) amine, glycol, glycerol, sorbitol, polyethylene glycol (PEG), salt form (sodium potassium, lithium), THAM (2-amino-2-hydroxymethyl-1,3-propanediol), and a pharmaceutically acceptable salt thereof; wherein X is absent or is selected from the group consisting of carbonyl,

methylene, oxygen, sulfur and nitrogen;

wherein Y is selected from the group consisting of methylene, methyl alkenyl, methylene alkynyl, methyleneoxycarbonyl, vinyl, and a C1 to C6 alkynyl; and wherein Z is a toxophore.

- 5 In one aspect, the toxophore Z is selected from the group consisting of 1-fluoro-1-carbonylmethyl and 1-nitro-2-carbonylethyl. In another aspect, Z is selected from the group consisting of doxorubicin, bis-(2-chloroethyl) amine, mitomycin, trichlorcarban, trichlorocarbanilide, tribromosalicylanilide, sulfamethoxazole, chloramphenicol, cycloserine, trimethoprim, chlorhexidine,
- 10 hexachlorophene, 2-mercaptopyridine-N-oxide, camptothecin, apoptolidene, cis-platin, anthracycline, epothilone, halichondrin, hemiasterlin, methioprim, thapsigargin, and fentichlor. In a further aspect, the toxophore Z is a chloro-substituted phenol. These include, but are not limited to the group consisting of 5-chloro-2-(2,4-dichlorophenoxy)phenol, 4-chloro-2-(2,4-dichlorophenoxy)phenol,
- 15 3-chloro-2-(2,4-dichlorophenoxy)phenol, 6-chloro-2-(2,4-dichlorophenoxy)phenol, 5-chloro-2-(3,4-dichlorophenoxy)phenol, 5-chloro-2-(2,5-dichlorophenoxy)phenol, and 5-chloro-2-(3,5-dichlorophenoxy)phenol. Alternatively, the toxophore Z is 2,2'-dihydroxy biphenyl ether. In a yet further embodiment, the toxophore Z is a halogenated 2-hydroxybenzophenone.

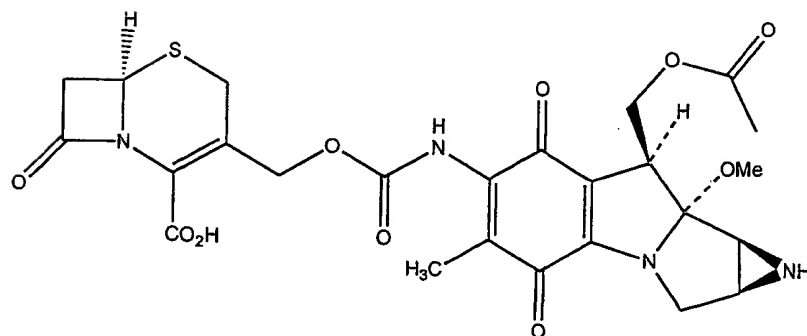
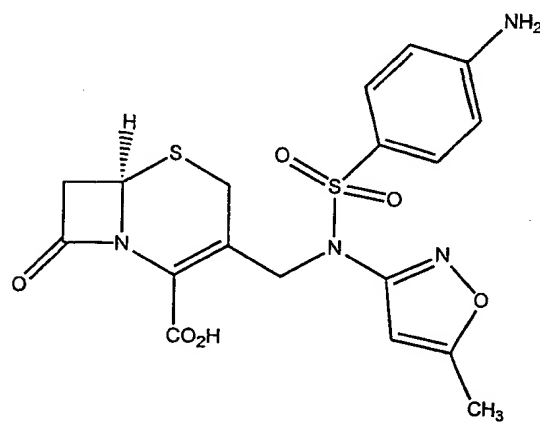
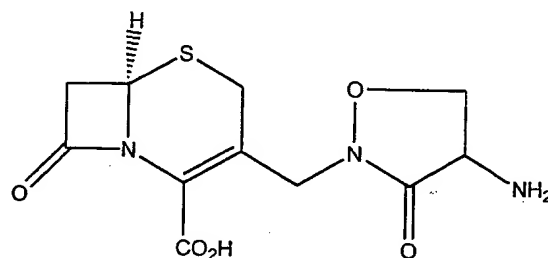
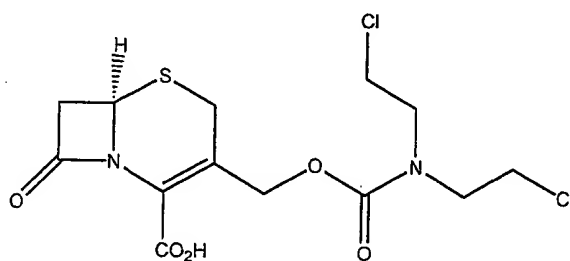
- 20 In a further embodiment, Y and X in combination is a substituent having a

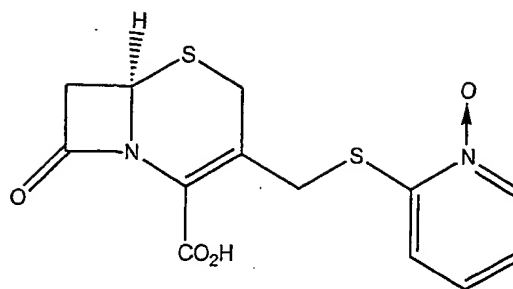
structure selected from the group consisting of ,  and  and Z

wherein T is selected from the group consisting of oxygen, nitrogen, sulphur and carbon.

- Specific embodiments of the invention, include, but are not limited to the
- 25 following modifications of the structure noted above:

1. Z is absent;
2. Y is a C2 to C3 alkynyl;
3. X is carbonyl or methylene;
4. X is methylene; and
- 30 5. Prodrugs having the structures:





Any of the compounds described herein can be combined with a carrier, e.g., a pharmaceutically acceptable carrier. This invention also provides a composition comprising the prodrug compounds as described above, alone or in combination with other compounds or other agents, known or yet to be discovered, and a carrier. In one embodiment, the carrier is a pharmaceutically acceptable carrier.

This invention also provides an *in vitro* method for assaying for drugs that inhibit or kill antibiotic resistant microorganisms, comprising the steps of contacting the drug with an antibiotic resistant microorganisms and separately contacting the antibiotic resistant microorganism with a prodrug compound of this invention and comparing the growth of the microorganisms, thereby assaying for drugs that inhibit or kill antibiotic resistant microorganisms. Drugs with activity to inhibit or kill antibiotic resistant microorganisms similar to the compounds of this invention are considered therapeutically relevant for further testing and development.

The method is particularly suited to assay for drugs that are effective against β -lactam or vancomycin-resistant microorganisms. The β -lactam resistant microorganism is a Gram-negative or a Gram-positive bacterium. Examples of such include, but are not limited to the Gram-negative bacteria selected from the group consisting of *Neisseria*, *Moraxella*, *Campylobacter*, *Enterobacteriaceae*, *Pseudomonas*, *Acinetobacter*, *Haemophilus* and *Bacteroides* and the Gram-positive bacteria selected from the group consisting of *Staphylococcus aureus*, *Staphylococcus epidermis* and other coagulase-negative staphylococci, *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Streptococcus agalactiae*, and *Enterococcus*.

This invention also provides a method for inhibiting the growth of an antibiotic resistant microorganism by contacting the microorganism with an effective amount of a prodrug compound of this invention. Contacting can be performed *in vitro* or *in vivo*. When contacted *in vitro*, the method provides a
5 means for controlling the growth of antibiotic resistant microorganisms on surfaces and for use as a disinfectant. *In vivo*, the method provides a positive control for animal models to test potential new drugs.

Varying concentrations of the potential agent are contacted with the sample to determine the optimal effective concentration of the agent. Thus, in one aspect,
10 this invention relates to the discovery and use thereof of agents that are selective substrates for enzymes that confer drug resistance to microorganisms.

Also provided by this invention are kits containing the prodrugs as described herein and instructions necessary to perform the screen.

Samples of cells or tissues as used herein encompass cells or tissues
15 characterized by the presence of drug resistance, the drug resistance being the result of the overexpression of an enzyme by the infecting microorganism. The cell can be a eucaryotic cell, i.e., a mammalian cell, e.g. a mouse cell, a rat cell, a hamster cell, or a human cell. The cell also can be a procaryotic cell such as a bacterial cell. The cell can be continuously cultured or isolated from an infected
20 animal or human subject.

The method can be practiced *in vitro*, *ex vivo* or *in vivo*. *In vivo* practice of the invention in an animal such as a rat or mouse provides a convenient animal model system that can be used prior to clinical testing of the therapeutic agent or prodrug. In this system, a potential prodrug will be successful if microbial load is
25 reduced or the symptoms of the infection are ameliorated, each as compared to an untreated, infected animal. It also can be useful to have a separate negative control group of cells or animals which has not been infected, which provides a basis for comparison.

When practiced *in vivo*, the candidate prodrug is administered to the
30 animal in effective amounts. As used herein, the term "administering" or "delivering" for *in vivo* and *ex vivo* purposes (if the target cell population is to be returned to the same (autologous) or another patient (allogeneic)) means providing

the subject with an effective amount of the candidate prodrug effective to reduce bacterial load. In these instances, the agent or prodrug may be administered with a pharmaceutically acceptable carrier. The agents, prodrugs and compositions of the present invention can be used in the manufacture of medicaments and for the treatment of humans and other animals by administration in accordance with conventional procedures, such as an active ingredient in pharmaceutical compositions.

Methods of administering pharmaceutical compositions are well known to those of ordinary skill in the art and include, but are not limited to, microinjection, intravenous or parenteral administration. The compositions are intended for topical, oral, or local administration as well as intravenously, subcutaneously, or intramuscularly. Administration can be effected continuously or intermittently throughout the course of the treatment. Methods of determining the most effective means and dosage of administration are well known to those of skill in the art and will vary with the prodrug used for therapy, the purpose of the therapy, the microorganism being treated, the severity of the infection, and the subject being treated. Single or multiple administrations can be carried out with the dose level and pattern being selected by the treating physician. For example, the compositions can be administered to a subject already suffering from an antibiotic resistant bacterial infection. In this situation, an effective "therapeutic amount" of the composition is administered to prevent continued and to at least partially arrest microbial growth and proliferation and ameliorate the symptoms associated with an infection.

However, the prodrugs can be administered to subjects or individuals susceptible to or at risk of developing an infection. In these embodiments, a "prophylactically effective amount" of the composition is administered to maintain cell viability and function at a level near to the pre-infection level.

It should be understood that by preventing or inhibiting unwanted cell death in a subject or individual, the prodrug compositions and methods of this invention also provide methods for treating, preventing or ameliorating the symptoms associated with a disease characterized by unwanted infection. Such

diseases include, but are not limited to the Gram-negative and Gram-positive infections, shown in the table below.

Organism	Disease(s)
GRAM-POSITIVE	
<i>Staphylococcus aureus</i>	Major human pathogen, bacteremia, pneumonia
<i>Staphylococcus epidermidis</i> and other coagulase-negative staphylococci	Urinary tract infections, osteomyelitis, bacteremia
<i>Streptococcus pyogenes</i>	Bacteremia, lymphangitis, pneumonia
<i>Streptococcus pneumoniae</i>	Pneumonia, otitis media, sinusitis
<i>Streptococcus agalactiae</i>	Primary bacteremia, pneumonia, endocarditis, osteomyelitis
<i>Enterococcus</i> species	Urinary tract infections, bacteremia, endocarditis, intra-abdominal and pelvic infections, neonatal sepsis
GRAM-NEGATIVE	
<i>Neisseria gonorrhoeae</i>	Genital infection, perihepatitis
<i>Moraxella catarrhalis</i>	Otitis media, lower respiratory tract infections, pneumonia, bacteremia
<i>Campylobacter jejuni</i>	Acute enteritis, acute colitis, bacteremia
Enterobacteriaceae (includes <i>Escherichia</i> , <i>Salmonella</i> , <i>Shigella</i> , <i>Klebsiella</i> , <i>Enterobacter</i>)	Enteric infections, urinary tract infections, respiratory infections, bacteremia, bacillary dysentery
<i>Pseudomonas aeruginosa</i>	Endocarditis, respiratory infections, bacteremia, central nervous system infections
<i>Acinetobacter</i> species	Respiratory tract infections, bacteremia, genitourinary
<i>Haemophilus influenzae</i>	Pneumonia, meningitis, epiglottitis, bacteremia
<i>Bacteroides</i>	Peritonitis

Amplification of genes associated with microbial resistance can be
 5 detected and monitored by a modified polymerase chain reaction (PCR) as
 described in U.S. Patent No. 5,085,983. Alternative assays include enzyme
 activity assays (Miller, 1992; Spector et al., 1997) and via the polymerase chain
 reaction (Spector et al., 1997; Maher et al., 1995).

The method is particularly suited to assay for drugs that are effective

against β -lactam or vancomycin-resistant microorganisms. The β -lactam resistant microorganism is a Gram-negative or a Gram-positive bacterium. Examples of such include, but are not limited to, the Gram-negative bacteria selected from the group consisting of *Neisseria*, *Moraxella*, *Campylobacter*, *Enterobacteriaceae*,
5 *Pseudomonas*, *Acinetobacter*, *Haemophilus* and *Bacteroides* and the Gram-positive bacteria selected from the group consisting of *Staphylococcus aureus*, *Staphylococcus epidermis* and other coagulase-negative staphylococci, *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Streptococcus agalactiae*, and *Enterococcus*.

10 Additionally, the invention is effective against vancomycin resistant organisms. The compounds of the invention kill the bacteria with a different mechanism of action. These prodrug compounds were designed to have dual modes of function. They can kill bacteria by the formation of bactericide in β -lactamase producing strains. They may also have cidal activity against non β -
15 lactamase strains by the mechanism of inhibiting cell wall biosynthesis. These compounds have enhanced activity against β -lactamase producing strains, as well as having potency against strains that lack β -lactamase. When these compounds are treated with the bacterial strains that lack β -lactamase they are expected to inhibit Penicillin binding protein (PBP), similar to conventional β -lactam
20 antibiotics. Meanwhile, equal-molar of bactericide is formed, thus producing the bactericidal activity. Therefore, for β -lactamase negative infectives, the compounds of the present invention exert their antibacterial activity by formation of bactericidal agents and also by inhibition of PBP.

Further provided by this invention is a method for treating a subject
25 infected with an antibiotic resistant microorganism by delivering to the subject an effective amount of a compound of this invention. The compound can be delivered as is or as a composition comprising a pharmaceutically acceptable carrier. As used herein, subject includes but is not limited to plants and vertebrates such as fish, mammals or avians, as defined above. The method is
30 particularly suited to assay for drugs that are effective against β -lactam or vancomycin-resistant microorganisms. The β -lactam resistant microorganism is a

Gram-negative or a Gram-positive bacterium. Examples of such include, but are not limited to the Gram-negative bacteria selected from the group consisting of Neisseria, Moraxella, Campylobacter, Enterobacteriaceae, Pseudomonas, Acinetobacter, Haemophilus and Bacteroides and the Gram-positive bacteria
5 selected from the group consisting of Staphylococcus aureus, Staphylococcus epidermis and other coagulase-negative staphylococci, Streptococcus pyogenes, Streptococcus pneumoniae, Streptococcus agalactiae, and Enterococcus.

When an effective amount of a compound or composition of this invention is administered to a subject, such as a plant, animal or human patient, an infection
10 can be treated or prevented.

The prodrug compounds of this invention also are useful for the manufacture of a medicament to treat antibiotic resistant microbial infections, e.g., β -lactam or vancomycin-resistant microorganisms. The β -lactam resistant microorganism can be a Gram-negative or a Gram-positive bacterium. Examples
15 of such include, but are not limited to the Gram-negative bacteria selected from the group consisting of Neisseria, Moraxella, Campylobacter, Enterobacteriaceae, Pseudomonas, Acinetobacter, Haemophilus and Bacteroides and the Gram-positive bacteria selected from the group consisting of Staphylococcus aureus, Staphylococcus epidermis and other coagulase-negative staphylococci,
20 Streptococcus pyogenes, Streptococcus pneumoniae, Streptococcus agalactiae, and Enterococcus.

In addition, the invention provides a method for selecting for antibiotic sensitivity, e.g., β -lactam activity, since a likely mechanism by which organisms gain resistance to the prodrugs is via loss of β -lactamase activity, which makes the
25 bacteria sensitive to β -lactam antibiotics once again. Thus, this invention provides a method of reversing antibiotic resistance in a microorganism by selecting for loss of the activity of the resistance enzyme. The method requires contacting the microorganism with a prodrug of this invention thereby killing the microorganisms expressing this enzyme. Using β -lactam as an example only, the
30 organisms having lost the β -lactamase enzyme will survive. These surviving organisms now are selected for sensitivity to the original antibiotic and can be effectively killed by contacting them with this antibiotic. Thus, the invention also

provides a combination therapy for the treatment of microbial infections, wherein the microorganism is capable of developing antibiotic resistance as defined below. The combination therapy requires first treating with the β -lactam antibiotic, then treating with a β -lactam prodrug as defined herein, and then finally, treating with the original β -lactam antibiotic. Also disclosed is a method for reversing
5 antibiotic resistance in a microorganism by contacting the microorganism with an effective amount of a prodrug of this invention.

Unlike the previous work (Melton & Sherwood, 1996), the prodrugs of the present invention need not be combined with a targeting agent. Thus, the
10 prodrugs can be directly utilized, topically or systemically.

This invention also provides a method for selectively inhibiting the proliferation of an antibiotic resistant microorganism, by contacting the microorganism with an effective amount of a prodrug of this invention. As noted above, the contacting can be accomplished *in vitro* against cultured or sampled cell
15 samples, *ex vivo*, or *in vivo* in an animal system. The methods of this invention also can be practiced *ex vivo* using a modification of the method described in U.S. Patent No. 5,399,346.

The prodrugs of this invention are useful to inhibit the proliferation of a microorganism that is resistant to a β -lactam antibiotic, e.g., penicillin or
20 cephalosporin. Additionally, the prodrugs of this invention are useful to inhibit the proliferation of a microorganism that is resistant to vancomycin.

β -lactamases can be found either extracellularly or within the periplasmic space of the microorganism. Genetic information for β -lactamase synthesis either can be carried on a plasmid or can occur within the bacterial chromosome; either of
25 these can result in the production of enzymes leading to resistance to the common β -lactam antibiotics.

Plasmid-mediated β -lactamases are especially insidious because of the ease with which these extrachromosomal elements can be transferred from one bacterial strain to another. Some β -lactamases, initially coded for on a plasmid, can have this
30 genetic information eventually incorporated into the chromosome as a permanent addition to the cellular deoxyribonucleic acid. It is not unusual for bacteria to carry

multiple plasmids, coding for multiple antibiotic-modifying enzymes. It is also possible that multiple resistance factors can be carried on a single plasmid. Thus, it is becoming common for bacteria to appear with resistance to two or three classes of antibiotics.

5 One of the most troubling aspects of chromosomal β -lactamase production is the ease of inducibility of these enzymes, resulting in high concentrations of β -lactamase. The best inducers known are β -lactam antibiotics, frequently those that are subsequently hydrolyzed by the induced enzyme. In some cases a stably repressed mutant may be selected, with total β -lactamase content representing as
10 much as 4% of the total protein in the bacterial cell.

 It is one of the aims of this invention to provide prodrugs that can be activated by any β -lactamase, thereby avoiding the problem of selecting the proper β -lactamase inhibitor. Because the β -lactam adduct of the prodrug will be broadly activated by β -lactamases of many species of bacteria (see, e.g., Vruthula
15 et al., 1995), a single prodrug will find utility for treating many different kinds of infections, previously resistant to treatment because of high levels of β -lactamase production by the target organism. This approach avoids the problem of mutation resistance encountered with β -lactamase inhibitors (Bush, 1988). This approach is also useful because resistance to these prodrugs is likely to come about via the
20 loss of β -lactamase activity. This will result in the bacterium regaining sensitivity to the penicillins. This invention therefore also claims a method for causing β -lactam antibiotic resistant organisms to become sensitive to β -lactam antibiotics by contacting the organism with an effective amount of a prodrug of this invention or an agent identified by the screen described above.

25 Another limitation of some currently available potent antibiotics is their lack of specificity. Examples include and doxorubicin, both isolated from *Streptomyces*. One of the major challenges in drug discovery and development is efficient targeting of the drug to a disease mechanism, with lack of effect on non-diseased, or host organs. Because many of the antibiotics that have been
30 discovered to date do not have good discrimination between bacterial and host targets, they have not been employed as anti-infective agents. Some of these

compounds have, however, been employed to treat other diseases, such as cancer. This invention provides a means of targeting these toxic compounds (in the form of the prodrug) to the infectious organism with minimal exposure of the host to the toxin.

5 Also relevant to this invention is the considerable prior art in which prodrug constructs of these antibiotics have been designed in which they are activated by bacterial specific enzymes, such as β -lactamase. In this technology, known as antibody directed prodrug therapy (ADEPT) or gene directed prodrug therapy (GDEPT), a bacterial enzyme is localized to a tumor via a specific
10 targeting agent, such as an antibody (Melton & Sherwood, 1996). The prodrug is then administered to the patient, and is activated preferentially at the tumor site (where the enzyme has been localized via its conjugation to antibody). This provides a localization of the antitumor antibiotic, allowing higher concentrations of the active drug at the tumor site, and less systemic exposure to the active drug
15 and its toxic activities. Several prodrugs have been prepared which are broadly activated by β -lactamases. These include β -lactam derivatives of doxorubicin (Vrudhula et al., 1995), paclitaxel (Rodrigues et al., 1995), nitrogen mustards (Kerr et al., 1995), vinca alkaloids (Meyer et al., 1992), and mitomycin (Vrudhula et al., 1995). These compounds have been shown to be activated by a broad
20 spectrum of β -lactamases from different bacterial species (Vrudhula et al., 1995). The effectiveness of these drugs is dependent upon proper localization of the activating enzyme via antibody binding to tumor, or by preferential expression of the activating enzyme in tumor cells. The authors of the publications cited above do not disclose the utility of the prodrugs as anti-infectious agents. This invention
25 requires no such localization by antibody or other methods because only the infectious organism is expressing the activating enzyme.

It is the purpose of the current invention to take advantage of this prodrug technology to treat infectious disease, not cancer. Similar prodrugs, activated by β -lactamase or other microbial enzymes normally involved in resistance to
30 antibiotic therapy, either expressed solely by the infectious agent and/or overexpressed by the infectious agent, will be used to activate prodrug versions of normally host-toxic drugs specifically to treat infections. This "biochemical

targeting" technology overcomes the lack of specificity of action of the drugs described above by having the activated forms created at high concentrations only within or at the sites of infectious disease. This is a novel approach and enables the use of drugs previously too toxic for use against infectious disease.

5 Previously, Mobashery and colleagues (Mobashery and Johnson, 1986) described a polypeptide antibiotic that appeared to be activated by β -lactamase. Their work failed to address several important issues: (1) Activity was dependent not only on β -lactamase expression, but also upon transport of the peptide into the bacterial cell by peptide permeases, and subsequent intracellular activation by other cellular
10 enzymes; (2) The peptide used was not active in an "enriched" medium (Boisvert et al., 1986). The first limitation means that access of the drug, and subsequent efficacy, was limited by its ability to enter the cell. Secondly, the peptide lacked activity in enriched medium, which is likely to be the situation encountered in any *in vivo* application. The authors do not anticipate enabling the use of the more
15 toxic antibiotics (such as mitomycin or doxorubicin) as a result of this "targeting" approach. Similarly, the groups working on ADEPT, while recognizing value in the application of β -lactam prodrugs to treat cancer, did not recognize their use in infectious disease.

Administration *in vivo* can be effected in one dose, continuously or
20 intermittently throughout the course of treatment. Methods of determining the most effective means and dosage of administration are well known to those of skill in the art and will vary with the composition used for therapy, the purpose of the therapy, the target cell being treated, and the subject being treated. Single or multiple administrations can be carried out with the dose level and pattern being
25 selected by the treating physician. Suitable dosage formulations and methods of administering the agents can be found below.

The pharmaceutical compositions can be administered orally, intranasally, parenterally or by inhalation therapy, and may take the form of tablets, lozenges, granules, capsules, pills, ampoules, suppositories or aerosol form. They may also
30 take the form of suspensions, solutions and emulsions of the active ingredient in aqueous or nonaqueous diluents, syrups, granulates or powders. In addition to an agent of the present invention, the pharmaceutical compositions can also contain

other pharmaceutically active compounds or a plurality of compounds of the invention.

More particularly, an agent of the present invention also referred to herein as the active ingredient, may be administered for therapy by any suitable route including oral, rectal, nasal, topical (including transdermal, aerosol, buccal and sublingual), vaginal, parental (including subcutaneous, intramuscular, intravenous and intradermal) and pulmonary. It will also be appreciated that the preferred route will vary with the condition and age of the recipient, and the disease being treated.

Ideally, the agent should be administered to achieve peak concentrations of the active compound at sites of disease. This may be achieved, for example, by the intravenous injection of the agent, optionally in saline, or orally administered, for example, as a tablet, capsule or syrup containing the active ingredient. Desirable blood levels of the agent may be maintained by a continuous infusion to provide a therapeutic amount of the active ingredient within disease tissue. The use of operative combinations is contemplated to provide therapeutic combinations requiring a lower total dosage of each component agent than may be required when each individual therapeutic compound or drug is used alone, thereby reducing adverse effects.

While it is possible for the agent to be administered alone, it is preferable to present it as a pharmaceutical formulation comprising at least one active ingredient, as defined above, together with one or more pharmaceutically acceptable carriers therefor and optionally other therapeutic agents. Each carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not injurious to the patient.

Formulations include those suitable for oral, rectal, nasal, topical (including transdermal, buccal and sublingual), vaginal, parenteral (including subcutaneous, intramuscular, intravenous and intradermal) and pulmonary administration. The formulations may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. Such methods include the step of bringing into association the active ingredient with the carrier that constitutes one or more accessory ingredients. In general, the

formulations are prepared by uniformly and intimately bringing into association the active ingredient with liquid carriers or finely divided solid carriers or both, and then if necessary shaping the product.

Formulations of the present invention suitable for oral administration may
5 be presented as discrete units such as capsules, cachets or tablets, each containing a predetermined amount of the active ingredient; as a powder or granules; as a solution or suspension in an aqueous or non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion. The active ingredient may also be presented a bolus, electuary or paste.

10 A tablet may be made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active ingredient in a free-flowing form such as a powder or granules, optionally mixed with a binder (e.g., povidone, gelatin, hydroxypropylmethyl cellulose), lubricant, inert diluent, preservative, disintegrant
15 (e.g., sodium starch glycolate, cross-linked povidone, cross-linked sodium carboxymethyl cellulose) surface-active or dispersing agent. Molded tablets may be made by molding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent. The tablets may optionally be coated or scored and may be formulated so as to provide slow or controlled release of the
20 active ingredient therein using, for example, hydroxypropylmethyl cellulose in varying proportions to provide the desired release profile. Tablets may optionally be provided with an enteric coating, to provide release in parts of the gut other than the stomach.

Formulations suitable for topical administration in the mouth include
25 lozenges comprising the active ingredient in a flavored basis, usually sucrose and acacia or tragacanth; pastilles comprising the active ingredient in an inert basis such as gelatin and glycerin, or sucrose and acacia; and mouthwashes comprising the active ingredient in a suitable liquid carrier.

Pharmaceutical compositions for topical administration according to the
30 present invention may be formulated as an ointment, cream, suspension, lotion, powder, solution, past, gel, spray, aerosol or oil. Alternatively, a formulation may

comprise a patch or a dressing such as a bandage or adhesive plaster impregnated with active ingredients and optionally one or more excipients or diluents.

If desired, the aqueous phase of the cream base may include, for example, at least about 30% w/w of a polyhydric alcohol, i.e., an alcohol having two or
5 more hydroxyl groups such as propylene glycol, butane-1,3-diol, mannitol, sorbitol, glycerol and polyethylene glycol and mixtures thereof. The topical formulations may desirably include a compound that enhances absorption or penetration of the agent through the skin or other affected areas. Examples of such dermal penetration enhancers include dimethylsulfoxide and related
10 analogues.

The oily phase of the emulsions of this invention may be constituted from known ingredients in a known manner. While this phase may comprise merely an emulsifier (otherwise known as an emulgent), it desirably comprises a mixture of at least one emulsifier with a fat or an oil or with both a fat and an oil.
15 Preferably, a hydrophilic emulsifier is included together with a lipophilic emulsifier that acts as a stabilizer. It is also preferred to include both an oil and a fat. Together, the emulsifier(s) with or without stabilizer(s) make up the so-called emulsifying wax, and the wax together with the oil and/or fat make up the so-called emulsifying ointment base which forms the oily dispersed phase of the
20 cream formulations.

Emulgents and emulsion stabilizers suitable for use in the formulation of the present invention include Tween 60, Span 80, cetostearyl alcohol, myristyl alcohol, glyceryl monostearate and sodium lauryl sulfate.

The choice of suitable oils or fats for the formulation is based on achieving
25 the desired cosmetic properties, since the solubility of the active compound in most oils likely to be used in pharmaceutical emulsion formulations is very low. Thus the cream should preferably be a non-greasy, non-staining and washable product with suitable consistency to avoid leakage from tubes or other containers. Straight or branched chain, mono- or dibasic alkyl esters such as di-isoadipate,
30 isocetyl stearate, propylene glycol diester of coconut fatty acids, isopropyl myristate, decyl oleate, isopropyl palmitate, butyl stearate, 2-ethylhexyl palmitate or a blend of branched chain esters known as Crodamol CAP may be used, the last

three being preferred esters. These may be used alone or in combination depending on the properties required. Alternatively, high melting point lipids such as white soft paraffin and/or liquid paraffin or other mineral oils can be used.

Formulations suitable for topical administration to the eye also include eye
5 drops wherein the active ingredient is dissolved or suspended in a suitable carrier, especially an aqueous solvent for the agent.

Formulations for rectal administration may be presented as a suppository with a suitable base comprising, for example, cocoa butter or a salicylate.

Formulations suitable for vaginal administration may be presented as
10 pessaries, tampons, creams, gels, pastes, foams or spray formulations containing in addition to the agent, such carriers as are known in the art to be appropriate.

Formulations suitable for nasal administration, wherein the carrier is a solid, include a coarse powder having a particle size, for example, in the range of about 20 to about 500 microns which is administered in the manner in which snuff
15 is taken, i.e., by rapid inhalation through the nasal passage from a container of the powder held close up to the nose. Suitable formulations wherein the carrier is a liquid for administration as, for example, nasal spray, nasal drops, or by aerosol administration by nebulizer, include aqueous or oily solutions of the agent.

Formulations suitable for parenteral administration include aqueous and
20 non-aqueous isotonic sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents, and liposomes or other microparticulate systems which are designed to target the compound to
25 blood components or one or more organs. The formulations may be presented in unit-dose or multi-dose sealed containers, for example, ampoules and vials, and may be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from
30 sterile powders, granules and tablets of the kind previously described.

Preferred unit dosage formulations are those containing a daily dose or unit, daily subdose, as herein above-recited, or an appropriate fraction thereof, of a agent.

It should be understood that in addition to the ingredients particularly
5 mentioned above, the formulations of this invention may include other agents conventional in the art having regard to the type of formulation in question, for example, those suitable for oral administration may include such further agents as sweeteners, thickeners and flavoring agents. It also is intended that the agents, compositions and methods of this invention be combined with other suitable
10 compositions and therapies.

These agents of this invention and the above noted compounds and their derivatives may be used for the preparation of medicaments for use in the methods described herein.

In the clinical use of the prodrug antibiotics will likely follow well
15 established guidelines. Dosage will likely be similar to those already employed for most other antibiotics. It is estimated that a dose of prodrug will be in the range of 100mg to 1 gm, given once every eight hours, or once a day, for one or two weeks, or until the patient tests negative for infectious organisms.

In one aspect, the invention encompasses a method of treating or
20 protecting plants from antibiotic resistant bacterial infections comprising applying an effective amount of the prodrug.

In order to achieve good dispersion and adhesion of the compounds as used to treat plants, it may be advantageous to formulate the compounds with components that aid dispersion and adhesion. Suitable formulations will be
25 known to those skilled in the art.

This invention also provides a method for treating or protecting plants from infection by bacteria resistant to antibiotics by applying an effective amount of the prodrug compound to the foliage, roots or the soil surrounding the plants or roots. These isolated compounds can be combined with known pesticides or
30 insecticides.

Compounds within the present invention when used to treat or protect plants from antibiotic resistant bacterial infections can be formulated as wettable

powders, granules and the like, or can be microencapsulated in a suitable medium and the like. Examples of other formulations include, but are not limited to soluble powders, wettable granules, dry flowables, aqueous flowables, wettable dispersible granules, emulsifiable concentrates and aqueous suspensions. Other
5 suitable formulations will be known to those skilled in the art.

This invention further provides a method for administering the prodrug compound to fish in an amount effective to either prevent or treat an antibiotic resistant bacterial infection. The compound may be administered by incorporating the compound into the food supply for the fish. Alternatively, the compound may
10 be added to the water in which the fish live, or are contained within. Finally, the compound may be administered to the fish as a suitable pharmaceutical preparation. Other suitable formulations will be known to those skilled in the art.

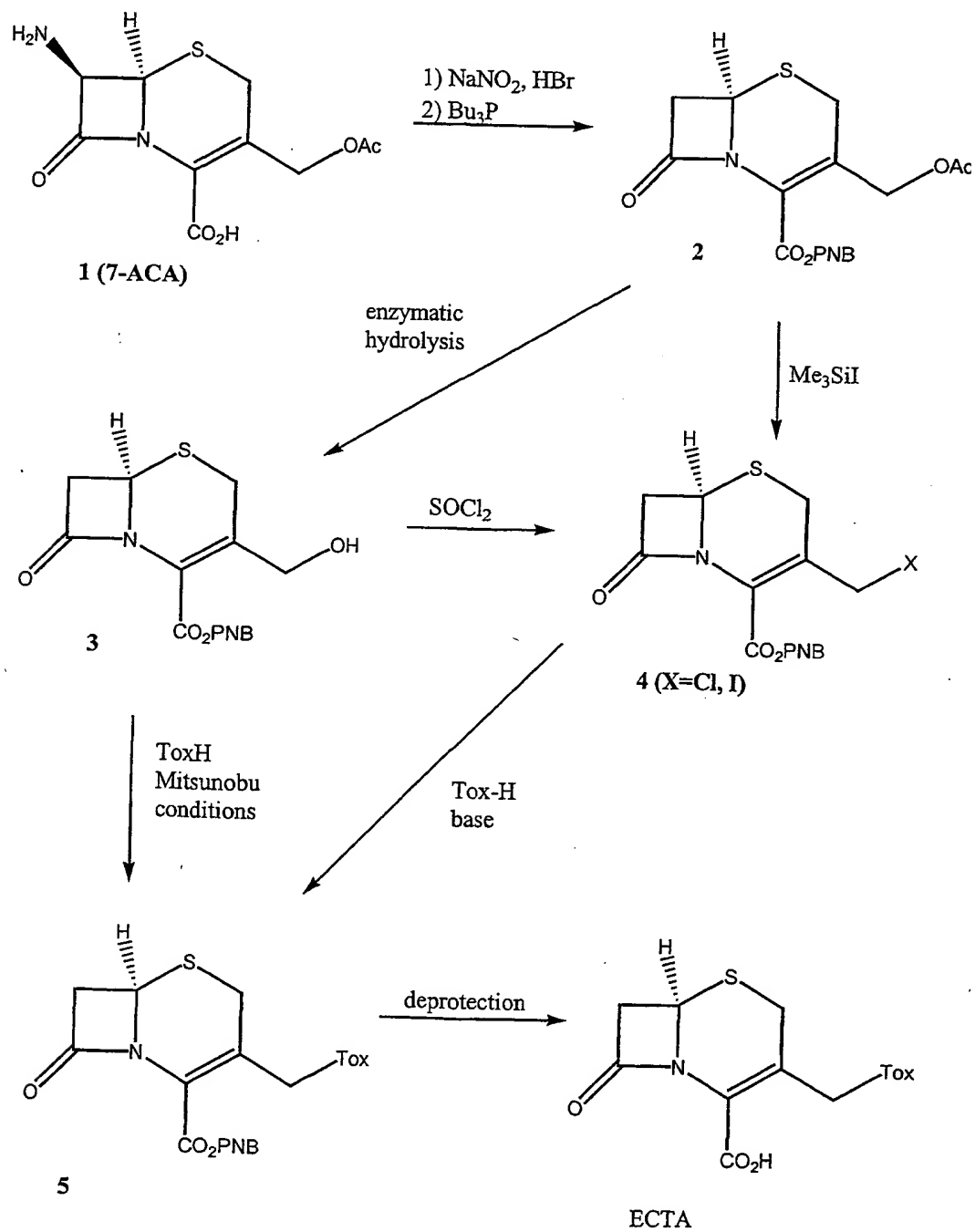
Materials and Methods

Processes for the Manufacture of the Prodrug Compounds

Further provided is a process for producing the prodrugs of this invention.

In general the process requires the following steps:

5



7- α -Bromocephalosporanic acid (2)

This compound is prepared according to the procedures of Rosati (US Patent No. 4,429,128, issued January 31, 1984) in 80% yield as an off white foam. It is used without purification in the next step.

5 **3-Acetoxymethylceph-3-em-4-carboxylic acid (3)**

7- α -Bromocephalosporinic acid is debrominated with tributylphosphine in MeOH using the procedure of Chern et al., 1988. A yellow foam is obtained in quantitative yield.

4-Nitrobenzyl 3-acetoxymethylceph-3-em-4-carboxylate (4)

10 The crude ceph-3-em-4-carboxylic acid obtained from above is dissolved in dimethylformamide (DMF). Di-isopropylethylamine (1 equivalent) is added dropwise under water cooling. 4-Nitrobenzyl bromide (1 equivalent) is added in small portions. The water bath was removed and the reaction mixture is stirred at ambient temperature for 4h. The solvent and volatile components are removed *in vacuo* and the residue is taken up in EtOAc. The organic solution is washed with
15 water, 0.5N HCl and saturated NaHCO₃ solution. After drying over anhydrous MgSO₄, the solvent is removed to give the crude product which is chromatographed on silica gel to give the product as an off white foam.

4-Nitrobenzyl 3-(iodomethyl)ceph-3-em-4-carboxylate (6)

20 The method of Bonjouklian, 1981, was used. To a solution of 4-nitrobenzyl cephalosporanate 4 in CH₂Cl₂ is added iodotrimethylsilane dropwise at 20° C under an atmosphere of nitrogen. The reaction is stirred at ambient temperature for 3h. The reaction mixture is diluted with CH₂Cl₂ and washed with 10% Na₂S₂O₃, brine and water, dried over MgSO₄ and evaporated *in vacuo* to give
25 the title compound as a light brown oil.

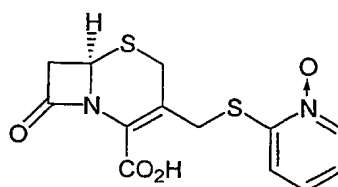
4-Nitrobenzyl 3-((5-chloro-2-(2,4-dichlorophenoxy)phenoxy)methyl)-ceph-3-em-4-carboxylate (7)

A mixture of the iodomethyl compound 6 (1 equivalent), triclosan (2,4,4'-trichloro-2'-hydroxydiphenyl ether, 1 equivalent), NaHCO₃ (1.2 equivalent) in
30 DMF is stirred at ambient temperature until all the starting material 6 is consumed as monitored by thin layer chromatography. The DMF is evaporated *in vacuo* and the residue was partitioned between EtOAc and water. The organic layer is

separated and washed with brine, dried over MgSO_4 and concentrated to give a crude product. Purification by column chromatography produces a white solid.

3-((5-chloro-2-(2,4-dichlorophenoxy)phenoxy)methyl)-ceph-3-em-4-carboxylic acid (8)

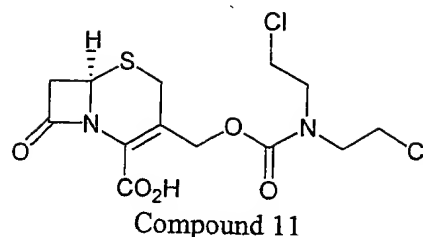
5 The 4-nitrobenzyl ester 7 is dissolved in MeOH. 5% Pd/C catalyst is added and the mixture is shaken under an atmosphere of hydrogen. When the reaction is complete, the catalyst is removed by filtration. The filtrate is concentrated under reduced pressure and taken up in EtOAc. The organic phase is extracted with saturated NaHCO_3 twice. The combined NaHCO_3 extracts is cooled to 0°C and
10 acidified (pH <2) with 1N HCl. The precipitated crystals are collected, washed with CH_2Cl_2 and dried *in vacuo* to give the desired product. Further purification is achieved by recrystallization.



Compound 10

3-(pyrid-2-yl-N-oxide)thiomethylceph-3-em-4-carboxylate (10)

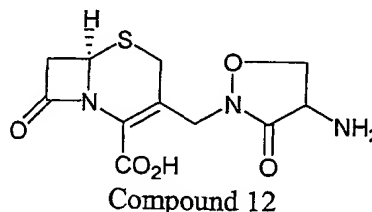
A mixture of the iodomethyl compound 6 (1.0 equivalent), 2-mercaptopyridine-N-oxide (1.0 equivalent), and NaHCO_3 (1.1 equivalent) in DMF
20 is stirred at ambient temperature until all the starting material 6 is consumed as monitored by thin layer chromatography. DMF is evaporated in vacuum and the residue is partitioned between EtOAc and water. The organic layer is separated, washed with brine, dried over MgSO_4 and concentrated to give a crude product. Purification by column chromatography gives a solid. Deprotection of the p-
25 nitrobenzyl group in the procedure described for compound 8 provided compound 10.



5 (11) **3-(N,N-bis(2-chloroethyl)carbamoyl)methylceph-3-em-4-carboxylate**

A solution of hydroxymethyl compound 3 (1.0 equivalent) and pyridine (1.0 equivalent) in anhydrous CH_2Cl_2 is cooled in an ice-bath and N,N-bis(2-chloroethyl)carbamoylchloride is slowly introduced via syringe. After 30 minutes
 10 the reaction is washed with water, brine, dried over MgSO_4 and concentrated to give crude product. Purification by column chromatography gives a solid. Deprotection of the p-nitrobenzyl group in the procedure described for compound 8 provides compound 11.

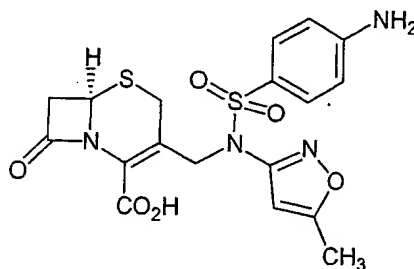
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20 (12) **3-(4-amino-3-oxo-isoxazolidin-2-yl)methyl ceph-3-em-4-carboxylate**

A mixture of hydroxymethyl compound 3 (1.0 equivalent) triphenylphosphine (1.0 equivalent), diethylazodicarboxylate (1.0 equivalent) and (3-Oxo-isoxazolidin-4-yl)-carbamic acid *tert*-butyl ester (1.0 equivalent) in
 25 anhydrous THF is stirred at ambient temperature for 4 hrs.

EtOAc is then added and the reaction mixture is washed with water, brine, dried over MgSO_4 . Concentration and purification by column chromatography gives a solid. Deprotection of the p-nitrobenzyl group in the procedure described for compound 8 provides compound 12.

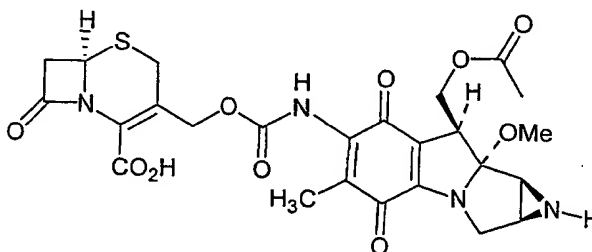


Compound 13

5 **3-[(4-Amino-benzenesulfonyl)-(5-methyl-isoxazol-3-yl)-amino]methyl cephalosporanic acid (13)**

A mixture of hydroxymethyl compound 3 (1.0 equivalent), triphenylphosphine (1.0 equivalent), diethylazodicarboxylate (1.0 equivalent) and sulfamethoxazole (1.0 equivalent) in anhydrous THF is stirred at ambient
10 temperature for 1 hr.

EtOAc is then added and the reaction mixture is washed with water, brine, dried over MgSO_4 . Concentration and purification by column chromatography gives a solid. Deprotection of the p-nitrobenzyl group in the procedure described for compound 8 provides compound 13.



Compound 14

15 **3-(Mitomycin carbamoyloxy)methyl cephalosporanic acid (14)**

20 A solution of hydroxymethyl compound 3 (1.0 equivalent), 2,6-lutidine (1.0 equivalent) in anhydrous THF is cooled in an ice-bath and 4-nitrophenylchloroformate is slowly added. After 30 minutes a solution of N-BOC mitomycin C in anhydrous THF is added under argon atmosphere. Ice-bath is then removed and stirring continued at ambient temperature until the completion of the
25 reaction as monitored by the thin layer chromatography. The reaction is then

washed with water, brine, and dried over MgSO_4 . Concentration and purification by column chromatography gives a solid. Deprotection of the p-nitrobenzyl group in the procedure described for compound 8 provides compound 14.

5 *In Vitro Assay*

Susceptibility testing is done by using the NCCLS (National Committee for Clinical Laboratory Standards) method to determine MIC's of antimicrobial compounds, modified for high-throughput screening. The MIC is defined as the lowest concentration at which bacteria growth (equivalent to visible growth) was inhibited after 16 to 18 hours of incubation at the appropriate temperature required for the bacteria growth. All stocks of tested compounds are prepared in either water or in DMSO, depending on the solubility. At the highest tested concentration, DMSO content should not exceed 0.5%. In brief, 20 2-fold serial dilutions of testing compounds from the highest concentration are made in a 384-well microtiter plate. Each well is inoculated with testing bacteria in broth to a final concentration of approximately $1-1.5 \times 10^6$ cells/ml. Bacterial growth is determined by the increase of optical density at 600 nm using a microplate reader (Tecan SpectraFluor Plus).

20 *Toxicity Assay*

Toxicity of the ECTA compound is determined by intravenously injecting groups of ICR-CD1 male mice (weighing approximately 22 to 25 grams) with various concentrations of the prodrug compound. The compound vehicle is used as control. Animals are observed twice a day for 14 days post-inoculation, and death is recorded. MTD (maximum tolerated dose) is determined.

In Vivo Assay

In vivo efficacy of the compound is evaluated by inoculating, intraperitoneally, ECR-CD1 mice with 0.5ml of bacteria at 100 times MLD. Mucin is used as the control. A single or multiple administration of the prodrug compound (either intravenously, subcutaneously, intramuscularly or oral) is administered post-inoculation. The compound vehicle is used as the control.

Animals are observed twice a day for 14 days post-inoculation, and death is recorded. ED50 (half effective dose) of the compounds are determined.

It is to be understood that while the invention has been described in conjunction with the above embodiments, that the foregoing description and the
5 following examples are intended to illustrate and not limit the scope of the invention. Other aspects, advantages and modifications within the scope of the invention will be apparent to those skilled in the art to which the invention pertains.

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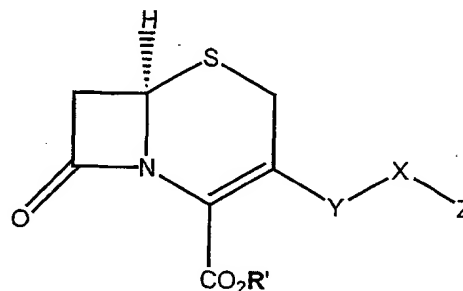
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CLAIMS

We claim:

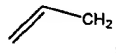
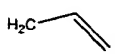

1. A prodrug compound having the structure:



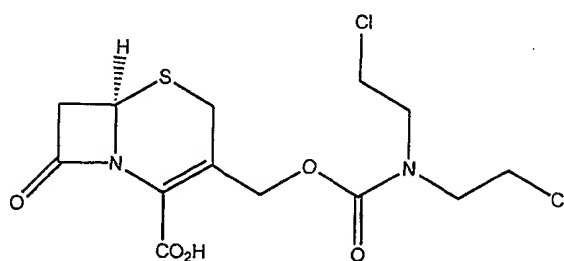
- wherein R' is selected from the group consisting of hydrogen, alkyl, aryl, halogenated aryl, phenol, nitro aryl, ammonium, methylamine, dimethylamine, lower alkylamine, bis (lower alkyl) amine, glycol, glycerol, sorbitol, polyethylene glycol (PEG), salt form (sodium, potassium, lithium), THAM (2-amino-2-hydroxymethyl-1,3-propanediol), and pharmaceutically acceptable salts thereof; wherein X is absent or is selected from the group consisting of carbonyl, methylene, oxygen, sulfur and nitrogen;
- 15 wherein Y is selected from the group consisting of methylene, methyl alkenyl, methylene alkynyl, methyleneoxycarbonyl, vinyl, and a C1 to C6 alkynyl; and wherein Z is a toxophore.

2. The compound of claim 1, wherein the toxophore Z is selected from the group consisting of 1-fluoro-1-carbonylmethyl and 1-nitro-2-carbonylethyl.
- 20

3. The compound of claim 1, wherein the toxophore Z is selected from the group consisting of doxorubicin, bis-(2-chloroethyl) amine, mitomycin, trichlorcarban, trichlorocarbanilide, tribromosalicylanilide, sulfamethoxazole, chloramphenicol, cycloserine, trimethoprim, chlorhexidine, hexachlorophene, 2-mercaptopyridine-N-oxide, camptothecin, apoptolidene, cis-platin, anthracycline, epothilone, halichondrin, hemicasterlin, methioprim, thapsigargin, and fenticlor.
- 25

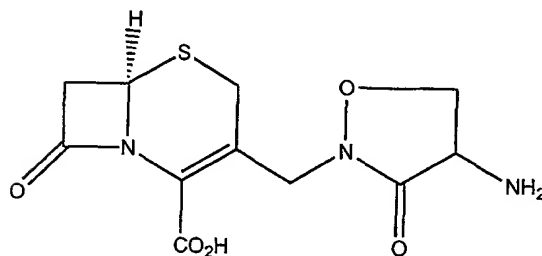
4. The compound of claim 1, wherein the toxophore Z is a chloro-substituted phenol.
5. The compound of claim 4, wherein the chloro-substituted phenol is selected from the group consisting of 5-chloro-2-(2,4-dichlorophenoxy)phenol, 4-chloro-2-(2,4-dichlorophenoxy)phenol, 3-chloro-2-(2,4-dichlorophenoxy)phenol, 6-chloro-2-(2,4-dichlorophenoxy)phenol, 5-chloro-2-(3,4-dichlorophenoxy)phenol, 5-chloro-2-(2,5-dichlorophenoxy)phenol, and 5-chloro-2-(3,5-dichlorophenoxy)phenol.
6. The compound of claim 1, wherein the toxophore Z is 2,2'-dihydroxy biphenyl ether.
7. The compound of claim 1, wherein the toxophore Z is a halogenated 2-hydroxybenzophenone.
8. The compound of claim 1, wherein Y and X in combination is a substituent having a structure selected from the group consisting of ,
-  and ,
- wherein T is selected from the group consisting of oxygen, nitrogen, sulphur and carbon.
9. The compound of any of claims 1 to 8, wherein Z is absent.
10. The compound of any of claims 1 to 8, wherein Y is a C2 to C3 alkynyl.
11. The compound of claim 1, wherein X is carbonyl or methylene.
12. The compound of claim 1, wherein X is methylene.

13. A prodrug compound having the structure:

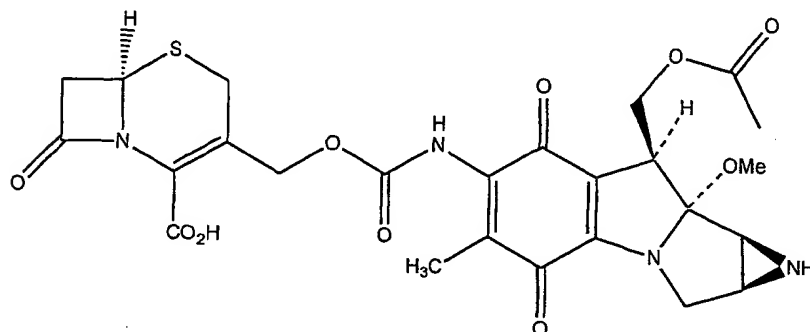


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14. A prodrug compound having the structure:

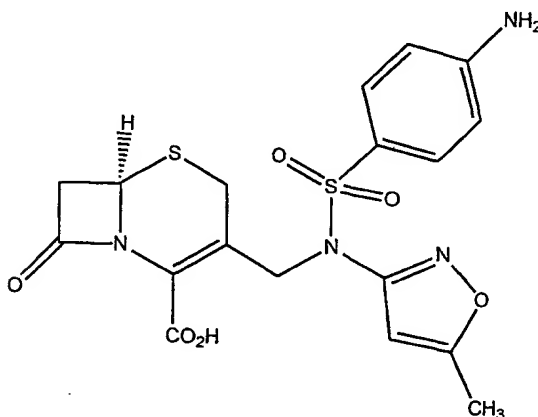


15. A prodrug compound having the structure:

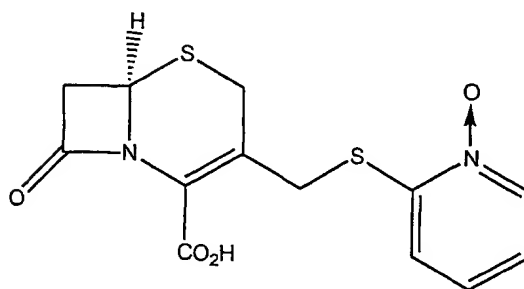


5

16. A prodrug compound having the structure:



17. A prodrug compound having the structure:



18. A composition comprising the compound of any of claims 1 to 8 or 11-17, and a carrier.

19. A composition comprising the compound of claim 9 and a carrier.
20. A composition comprising the compound of claim 10 and a carrier.
- 5 21. The composition of claim 18, wherein the carrier is a pharmaceutically acceptable carrier.
22. The composition of claim 19, wherein the carrier is a pharmaceutically
10 acceptable carrier.
23. The composition of claim 20, wherein the carrier is a pharmaceutically acceptable carrier.
- 15 24. An *in vitro* method for assaying for drugs that inhibit or kill antibiotic resistant microorganisms, comprising the steps of:
- a) contacting the drug with an antibiotic resistant microorganisms and separately contacting the antibiotic resistant microorganism with a compound of claim 1; and
 - 20 b) comparing the growth of the microorganisms, thereby assaying for drugs that inhibit or kill antibiotic resistant microorganisms.
- 25 25. The method of claim 24, wherein the antibiotic resistant microorganism is a β -lactam resistant microorganism.
26. The method of claim 24, wherein the antibiotic resistant microorganism is a vancomycin-resistant microorganism.
27. The method of claim 25, wherein the β -lactam resistant microorganism is a
30 Gram-negative bacterium.
28. The method of claim 27, wherein the Gram-negative bacteria is selected

from the group consisting of *Neisseria*, *Moraxella*, *Campylobacter*,
Enterobacteriaceae, *Pseudomonas*, *Acinetobacter*, *Haemophilus* and *Bacteroides*.

29. The method of claim 25, wherein the β -lactam resistant microorganism is a
5 Gram-positive bacterium.

30. The method of claim 29, wherein the Gram-positive bacteria is selected
from the group consisting of *Staphylococcus aureus*, *Staphylococcus epidermis*,
coagulase-negative staphylococci, *Streptococcus pyogenes*, *Streptococcus*
10 *pneumoniae*, *Streptococcus agalactiae*, and *Enterococcus*.

31. A method for inhibiting the growth of an antibiotic resistant
microorganism comprising contacting the microorganism with an effective
amount of the compound of claim 1.
15

32. The method of claim 31, wherein the antibiotic resistant microorganism is
a β -lactam resistant microorganism.

33. The method of claim 31, wherein the antibiotic resistant microorganism is
20 a vancomycin-resistant microorganism.

34. The method of claim 32, wherein the β -lactam resistant microorganism is a
Gram-negative bacterium.

25 35. The method of claim 34, wherein the Gram-negative bacteria is selected
from the group consisting of *Neisseria*, *Moraxella*, *Campylobacter*,
Enterobacteriaceae, *Pseudomonas*, *Acinetobacter*, *Haemophilus* and *Bacteroides*.

36. The method of claim 32, wherein the β -lactam resistant microorganism is a
30 Gram-positive bacterium.

37. The method of claim 36, wherein the Gram-positive bacteria is selected

from the group consisting of *Staphylococcus aureus*, *Staphylococcus epidermis*, coagulase-negative staphylococci, *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Streptococcus agalactiae*, and *Enterococcus*.

- 5 38. A method for treating a subject infected with an antibiotic resistant microorganism comprising delivering to the subject an effective amount of the compound of claim 1.
39. The method of claim 38, wherein the antibiotic resistant microorganism is
10 a β -lactam resistant microorganism.
40. The method of claim 38, wherein the antibiotic resistant microorganism is a vancomycin-resistant microorganism.
- 15 41. The method of claim 39, wherein the β -lactam resistant microorganism is a Gram-negative bacterium.
42. The method of claim 41, wherein the Gram-negative bacteria is selected from the group consisting of *Neisseria*, *Moraxella*, *Campylobacter*,
20 *Enterobacteriaceae*, *Pseudomonas*, *Acinetobacter*, *Haemophilus* and *Bacteroides*.
43. The method of claim 39, wherein the β -lactam resistant microorganism is a Gram-positive bacterium.
44. The method of claim 43, wherein the Gram-positive bacteria is selected from the group consisting of *Staphylococcus aureus*, *Staphylococcus epidermis*,
25 coagulase-negative staphylococci, *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Streptococcus agalactiae*, and *Enterococcus*.
45. The method of claim 38, wherein the subject is a plant.
46. The method of claim 38, wherein the subject is a vertebrate.
47. The method of claim 46, wherein the vertebrate is a fish, mammal or an

avian.

48. Use of a compound of any of claims 1 to 8 or 11 to 17 for the manufacture of a medicament to treat antibiotic resistant infections.
49. Use of a compound of claim 10 for the manufacture of a medicament to
5 treat antibiotic resistant infections.
50. Use of a compound of claim 11 for the manufacture of a medicament to treat antibiotic resistant infections.
51. The use of claim 48, wherein the antibiotic resistant microorganism is a β -lactam or vancomycin resistant microorganism.
- 10 52. The use of claim 49, wherein the antibiotic resistant microorganism is a β -lactam or vancomycin resistant microorganism.
53. The use of claim 50, wherein the antibiotic resistant microorganism is a β -lactam or vancomycin resistant microorganism.
54. A method for selecting for antibiotic sensitivity for reversing antibiotic
15 resistance in a microorganism expressing an enzyme that confers antibiotic resistance to the microorganism comprising contacting the microorganism with a compound of claim 1 thereby killing the microorganisms expressing this enzyme.
55. The method of claim 54, wherein the enzyme is β -lactamase.
56. A method for inhibiting the growth or killing a microorganism comprising
20 contacting the microorganism first with an antibiotic and subsequently contacting the microorganisms with a compound of claim 1.
57. A method for reversing antibiotic resistance in a microorganism by contacting the microorganism with an effective amount of a compound of claim 1.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/06519

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : C07D 501/00; A61K 31/545, 31/546; A61P 31/04
US CL : Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 540/215, 224, 226, 229, 230, 222; 514/202, 203, 204, 207, 209, 200; 435/32

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
CAS ONLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X — A	US 4,348,264 A (ROSATI) 07 September 1982, see col. 47, line 51.	1, 9 — 2-8, 10-57
X — A	RO 88451 A (INSTITUTUL DE CERCETARI CHIMICO-FARMACEUTICE) 30 January 1986, see col. 4, lines 31-33.	1 — 2-57

☐ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

* Special categories of cited documents:	* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
E earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*Z* document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means	
P document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

19 APRIL 2001

Date of mailing of the international search report

13 JUL 2001

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Form PCT/ISA/210 (second sheet) (July 1998) *

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/06519

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

540/215, 224, 226, 229, 230, 222; 514/202, 203, 204, 207, 209, 200; 435/32